Institute of Biodiversity and Environmental Conservation

Evaluation of Antimicrobial Compound Effect from *Senna alata* Linn. Against Multiple Antibiotic Resistance (MAR) Bacteria from Environment

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Master of Science
2024

Evaluation of Antimicrobial Compound Effect from *Senna alata* Linn. Against Multiple Antibiotic Resistance (MAR) Bacteria from Environment

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A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Microbiology)

Institute of Biodiversity and Environmental Conservation
UNIVERSITI MALAYSIA SARAWAK
2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

……

Signature

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Date : 1 August 2024

ACKNOWLEDGEMENT

All praise and thanks to God for the blessings, knowledge, courage, patience, and strength to complete my research until it succeeds.

I want to take this opportunity to express my utmost gratitude and appreciation to my supervisor, Associate Professor Dr. Samuel Lihan, for his constant guidance, encouragement, advice, and patience throughout the completion of this research.

I would also like to extend my gratitude to my colleagues from the Molecular Laboratory, Institute of Biodiversity and Environmental Conservation, UNIMAS and Department of Animal Science and Fisheries, UPM Bintulu Campus, for their support in providing test organisms for the research. I also like to thank all the laboratory assistants and technical staff for providing all the necessary materials, facilities, and equipment throughout this research.

My warmest thanks to my parents, Bunya Ingun and Jemurau Empam, and my siblings for their love, patience, prayer, support, and inspiration.

My special thanks to other postgraduate students, especially to Flonia bt Benet, Stanley Sait, Sia Siew Chuiang, Marshallanore Mhellus, Nurhaziqa bt Yazid, Sophia Suring Ann, and Claudia Lenya for their helpful cooperation and support towards completion of this study.

Finally, I would like to express my gratitude and appreciation to Universiti Malaysia Sarawak for the Zamalah scholarship. Thank you.

ABSTRACT

The increasing prevalence of multiple antibiotic-resistant (MAR) bacteria has increased the interest in exploring the potential of medicinal plants as a source of antimicrobial agents. *Senna alata* Linn. commonly known as "*gelenggang*" and "*daun kurap*" in Malaysia, is a medicinal plant with various ethnomedical uses and biological features. This study evaluated the antibacterial activity of leaves, stems, and roots against MAR bacteria from the environment, *Enterococcus* sp., and *Mammaliicoccus* sp. The bacteria used in this study were obtained from the environmental bacterial stock provided by the Molecular Laboratory, Institute of Biodiversity and Environmental Conservation, UNIMAS and Department of Animal Science and Fisheries, Universiti Putra Malaysia, Bintulu Campus. From the antibiotic susceptibility test, the MAR value *Enterococcus* sp. was 0.83, which showed resistance against erythromycin, tetracycline, norfloxacin, chloramphenicol and vancomycin. Meanwhile, in *Mammaliicoccus* sp., the MAR value was 0.40, and resistant towards piperacillin, aztreonam, ceftazidime and penicillin. The extraction methods used were soxhlet extraction (SE) and ultrasonic-assisted extraction (UAE), accompanied by ethanol, chloroform and hexane as the choice of solvent. Among all plant parts, the soxhlet and ethanol extracts produced higher yields. However, in the next assay, hexane was removed as it gave a very low yield and was not suitable as most assays needed a high extract concentration. On the other hand, leaves extract showed a greater yield than stems and roots. *S. alata* extracts were also tested for their total phenolic content (TPC) and total flavonoid content (TFC) using Folin-Ciocalteu and aluminium chloride methods, respectively. Both TPC and TFC were exhibited the highest by the leaves of ultrasonic and chloroform extract, UAE-Chlo-L (TPC=117.44±2.78 mg GAE/g DW, TFC=568.78±24.44 mg QE/g DW), followed by the leaves of soxhlet and chloroform extract, SE-Chlo-L. (TPC=80.39±6.60 mg

GAE/g DW, TFC=406.44±7.41 mg QE/g DW). In antibacterial activity using agar well diffusion assay, most of the extracts, especially from soxhlet extraction, were more sensitive and inhibited higher in the *Mammaliicoccus* sp. compared to *Enterococcus* sp. The largest inhibition towards *Mammaliicoccus* sp. was exhibited by SE-Chlo-S (15.00 ± 0.00 mm) with minimum inhibitory concentration and minimum bactericidal concentration at 0.39062 mg/mL and 3.125 mg/mL, respectively. Based on the minimum inhibitory concentration/minimum bactericidal concentration ratio conducted, all the extracts in this study showed potential as bactericidal agents. Besides, the extracts' bioactive compounds associated with antibacterial activity were identified using thin-layer chromatography-direct bioautography (TLC-DB) assay and gas chromatography-mass spectrometry (GC-MS) analysis. Among many spots identified on the TLC plates, only L2 (R_f = 0.47), S2 (R_f = 0.40,) and R1 (R_f =0.42) showed inhibition towards tested bacteria. Various bioactive compounds such as Phenol, 3,5-bis(1,1-dimethylethyl)-, Cholest-5-en-3-ol (3.beta.)-, carbonochloridate, Neophytadiene, Decane, 3,7-dimethyl-, Dodecane, 2,6,11-trimethyl-, 2-Pentadecanone, 6,10,14-trimethyl-, and many other compounds were identified. The findings in this study indicated that *S. alata* possessed antibacterial activities besides having unique bioactive compounds that can be used as a potential antibacterial agent for the production of new drugs in combatting the multiple antibiotic resistance issues in bacteria, particularly bacteria from the environment.

Keywords: *Senna alata* Linn., phenolic content, flavonoid content, antibacterial activity, bioactive compounds

Penilaian Kesan Kompaun Antimikrob dari Senna alata Linn. terhadap Bakteria Rintangan Antibiotik Berbilang (MAR) daripada Persekitaran

ABSTRAK

Peningkatan bakteria dengan perintang pelbagai antibiotik telah menaikkan minat untuk meneroka potensi tumbuhan ubatan sebagai sumber agen antimikrob. Sennaa alata Linn. atau lebih dikenali sebagai "gelenggang" dan "daun kurap" di Malaysia, merupakan tumbuhan ubatan dengan pelbagai kegunaan etnoperubatan dan ciri biologi. Kajian ini dijalankan untuk menilai aktiviti antibakteria daripada daun, batang dan akar terhadap bakteria dengan perintang pelbagai antibiotik, iaitu Enterococcus sp. dan Mammaliicoccus sp. Bakteria ujian yang digunakan dalam kajian ini diperoleh daripada stok bakteria persekitaran yang disediakan oleh Makmal Molekul, Institut Biodiversiti dan Pemuliharaan Alam Sekitar, UNIMAS dan Jabatan Sains Haiwan dan Perikanan, Universiti Putra Malaysia, Kampus Bintulu. Daripada ujian kerentanan antibiotik, nilai MAR Enterococcus sp. adalah 0.83, yang menunjukkan ketahanan terhadap eritromisin, tetrasiklin, norfloxacin, kloramfenikol dan vankomisin. Manakala, dalam Mammaliicoccus sp., nilai MAR ialah 0.40, dan tahan terhadap piperacillin, aztreonam, ceftazidime dan penisilin. Kaedah pengekstrakan yang digunakan ialah pengekstrakan sokhlet (SE) dan pengekstrakan berbantu ultrasonik (UAE), disertai etanol, kloroform dan heksana sebagai pelarut pilihan. Di antara semua bahagian tumbuhan, ekstrak soxhlet dan etanol menunjukkan pengeluaran hasil yang lebih tinggi. Walau bagaimanapun, dalam ujian seterusnya, heksana telah dikeluarkan kerana ia memberikan hasil yang sangat rendah, dan tidak sesuai kerana kebanyakan ujian memerlukan kepekatan ekstrak yang tinggi. Selain itu, ekstrak daun menunjukkan hasil yang lebih besar daripada batang dan akar.

Ekstrak S. alata juga diuji untuk mengenalpasti jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC) di mana masing-masing menggunakan kaedah Folin-Ciocalteu dan aluminium klorida. Kedua-dua nilai TPC and TFC telah dipamerkan tertinggi oleh ekstrak daripada daun yang menggunakan kaedah ultrasonik gabungan kloroform, UAE-Chlo-L (TPC=117.44±2.78 mg GAE/g DW, TFC=568.78±24.44 mg QE/g DW), diikuti oleh ekstrak daun daripada kaedah sokhlet gabungan kloroform, SE-Chlo-L. (TPC=80.39±6.60 mg GAE/g DW, TFC=406.44±7.41 mg QE/g DW). Dalam aktiviti antibakteria menggunakan ujian resapan telaga-agar, kebanyakan ekstrak, terutamanya daripada pengekstrakan sokhlet adalah lebih sensitif dan menghalang lebih tinggi dalam Mammaliicoccus sp., jika dibandingkan dengan Enterococcus sp. Zon perencatan terbesar terhadap Mammaliicoccus sp. telah dipamerkan oleh SE-Chlo-S (15.00 ± 0.00 mm) dengan nilai kepekatan perencatan minimum dan kepekatan bakteria minimum pada 0.39062 mg/mL dan 3.125 mg/mL, masing-masing. Berdasarkan nisbah nilai kepekatan perencatan minimum/kepekatan bakteria minimum yang dijalankan, kesemua ekstrak dalam kajian ini menunjukkan potensi sebagai agen bakteria. Selain itu, sebatian bioaktif yang dikaitkan dengan aktiviti antibakteria daripada ekstrak telah dikenal pasti menggunakan analisis kromatografi lapisan nipis -bioautografi langsung (TLC-DB) dan kromatografi gas - spektrometri jisim (GC-MS). Di antara banyak tompok yang dikenal pasti pada plat TLC, hanya L2 (Rf = 0.47), S2 (Rf = 0.40) dan R1 (Rf =0.42) menunjukkan perencatan terhadap bakteria yang diuji. Pelbagai sebatian bioaktif seperti Phenol, 3,5-bis(1,1-dimethylethyl)-, Cholest-5-en-3-ol (3.beta.)-, carbonochloridate, Neophytadiene, Decane, 3,7-dimethyl- , Dodecane, 2,6,11-trimethyl-, dan 2-Pentadecanone, 6,10,14-trimethyl-, dan banyak sebatian lain telah dikenal pasti. Penemuan dalam kajian ini menunjukkan bahawa S. alata mempunyai aktiviti antibakteria selain mempunyai sebatian bioaktif unik yang boleh

digunakan sebagai agen antibakteria yang berpotensi untuk pengeluaran ubat-ubatan baru dalam mengatasi pelbagai isu rintangan antibiotik dalam bakteria, terutamanya bakteria dari persekitaran.

Kata kunci: *Senna alata Linn., kandungan fenolik, kandungan flavonoid, aktiviti antibakteria, sebatian bioaktif*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ATTC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
CFU	Colony Forming Unit
Chlo	Chloroform
CLSI	Clinical and Laboratory Standard Institute
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DW	Dry Weight
EtOAc	Ethyl acetate
EtOH	Ethanol
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography-Mass Spectrometry
Hex	Hexane
kb	Kilobase
kHz	Kilohertz
mA	milliampere
MAR	Multiple Antibiotic Resistant
MBC	Minimum Bactericidal Concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

NASM	Non-aureus staphylococci and mammaliicocci
NB	Nutrient Broth
NCBI	National Center for Biotechnology Information
NIST	National Institute of Standards and Technology
nm	Nanometre
PCR	Polymerase Chain Reaction
Rf	Retention Factor
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
rRNA	Ribosomal Ribonucleic Acid
SE	Soxhlet Extraction
TFC	Total Flavonoid Content
TLC	Thin Layer Chromatography
TLC-DB	Thin Layer Chromatography – Direct Bioautography
TPC	Total Phenolic Content
UAE	Ultrasonic Assisted Extraction
UV	Ultra-violet
UV-Vis	Ultraviolet-Visible spectrophotometry
V	Volt

CHAPTER 1

INTRODUCTION

1.1 Study Background

Ethnopharmacological aspects of herbal medicine and the discovery of plant-based drugs play a significant role in the evolution of modern therapeutic systems (Süntar, 2020). In traditional practices, medicinal substances were made into herbal remedies with various compositions, including powders, tinctures, pastes, and teas, particularly to address specific health issues (Sreekumar & Nisha, 2022). As in research and development, isolating and identifying physiologically active chemicals and molecules from nature has resulted in the discovery of new treatments, thereby enhancing the health and pharmaceutical industries. These phytochemicals have the potential to serve as a reservoir of novel compounds, which can be utilised in the creation of new pharmaceutical medicines (Lautié et al., 2020).

The World Health Organisation (WHO) has compiled a comprehensive list of 21,000 plants widely utilised for their therapeutic properties worldwide (Anand et al., 2019). In Borneo, the tropical rainforests are an important source of medicinal plants. Its rich biodiversity leads to numerous ethnomedical practices (Baling et al., 2017). Many of the world's population gets their health care from traditional medicines. For example, the ethnic communities in Sarawak, including the Kedayan, Melanau, Bidayuh, Orang Ulu, and Iban, have utilised medicinal plants for various treatments for generations. The utilisation of herbal medicines, rooted in a knowledge of traditional medicine, has been passed across many generations (Bakar et al., 2023).

Senna alata Linn. (previously known as *Cassia alata*) is a native plant from the Fabaceae family with various therapeutic uses. This species is found in various tropical

countries, including Africa, America, India, Fiji, Indonesia, and Malaysia (Oladeji et al., 2020). It is known as "*gelenggang*" and "*daun kurap*" in Malaysia, whereas in Indonesia it is referred as "*ketepang badak*" (Fatmawati et al., 2020). Different parts from *S. alata* have been known to have therapeutic properties. Traditionally, *S. alata* has been utilised for centuries to treat a wide range of skin disorders in several Asian and African nations. Furthermore, it has registered as an herbal medication in Thailand and developed as a topical fungal skin infection treatment agent (Silalahi, 2022).

Fresh leaves of the plant are utilised to treat skin infections, mycosis, and dermatitis in Malaysia (Oladeji et al., 2020). The Muruts, Kedayan, and Kadazandusun of Borneo often used the leaves to treat skin disorders such as ringworm by pressing the pounded leaves into the infected area. Some use it as a tonic to treat yellowish skin and asthma (Kulip, 2005; Ahmad & Holdsworth, 1994 cited in Bakar et al., 2023). Besides, the leaf extract that initially demonstrated clinically significant antifungal activity against the yeast *Malassezia furfur* originated in the Philippines. The leaf decoction also appeared to have therapeutic potential for tinea imbricata, a fungal infection caused by *Trichophyton concentricum* (Eusebio-Alpapara et al., 2020). Aside from that, the leaf decoction was utilised to treat constipation in Thailand. Meanwhile, In China, the seeds are brewed into a beverage beneficial for asthma and eyesight (Yon et al., 2022).

The various ethnomedicinal benefits of *S. alata* have prompted researchers to analyse plant sources for bioactive compounds. This approach is considered a novel strategy for developing new pharmaceutical and biological resources in healthcare systems (Vaou et al., 2021; Rahman et al., 2022). Bioactive compounds are plants' most abundant secondary metabolites broadly distributed in plant sources. Plant secondary metabolites are not considered necessary for fundamental plant functions (Samtiya et al., 2021). Nevertheless,

both traditional and contemporary medicine rely extensively on these bioactive compounds. The chemical composition of these substances includes glycosides, flavonoids, tannins, terpenoids, lignans, alkaloids, peptides, and many other compounds. They possess significant natural antioxidant properties and are crucial sources of remedies and pharmaceuticals (Gürbüz et al., 2018).

Various studies have suggested that the bioactive compounds in *S. alata* possess a wide spectrum of biological properties such as anti-inflammatory, antioxidant, antidiabetic, antimicrobial, anticancer and antidepressant (Fatmawati et al., 2020). Phenolic compounds, alkaloids, anthraquinones, tannins, steroids and flavonoids were the common bioactive compounds in them. These compounds' presence correlates to the medicinal and biological properties reported in the plants (Adelowo & Oladeji, 2017).

1.2 Problem Statement

Bacteria have been discovered in the water system, aquaculture sectors, pharmaceutical industries, and food production throughout the last several years (González-Plaza et al., 2019; Peters et al., 2019). Multiple antibiotic resistant (MAR) bacteria were discovered in the environment even before the use of antibiotics in clinical, agricultural, and aquaculture settings (Serwecinska, 2020). The widespread use of antibiotics in many sectors has widened the pathways for the emergence and spread of resistant bacteria (Li & Webster, 2018; Serwecinska, 2020).

Low- and middle-income countries are more susceptible to antibiotic resistance. Hospital overcrowding, poor hygiene, a surge in meat and fish output, a lack of affordable second or third-line antibiotics, and a lack of surveillance and diagnostics all contribute to this problem (Laxminarayan et al., 2020). As antibiotic resistance grows, it restricts the efficacy of treatment for various bacterial, fungal, and viral infections. It also jeopardises the

ability to manage common diseases such as typhoid and influenza, resulting in protracted illness and treatment, lifelong impairment, or even death. Besides, patients with antibiotic-resistant infections require longer treatment and more expensive medications, which raises their healthcare expenses (Abushaheen et al., 2020).

Gram-negative bacterial pathogens are accorded a higher priority than Gram-positive bacteria because of their greater evolution and resistance mechanism levels. This gap can be seen in the surveillance and research on antibiotic resistance in Gram-positive bacteria (Osei Sekyere & Mensah, 2020). *Enterococcus* sp. is a type of Gram-positive bacteria present in various settings, including soil, water, and sewage, as well as the digestive tracts of humans, animals, and insects. While some *Enterococcus* sp. are commensal, others have the potential to be pathogens (Krawczyk et al., 2021).

Besides, *Mammaliicoccus* sp. is also recognised as Gram-positive bacteria. *Mammaliicoccus sciuri* (*M.* sciuri) is the type species of the novel genus *Mammaliicoccus*, which was originally *Staphylococcus sciuri* (Madhaiyan et al., 2020). While most *Mammaliicoccus* isolates are considered commensals, they can occasionally infect both people and animals. They may serve as a repository for resistance genes and were detected in both wild and farmed animals and the environment (Schwendener & Perreten, 2022).

Because of the increasing prevalence of MAR bacteria, researchers are particularly interested in the study of antimicrobial compounds derived from medicinal plants (Vaou et al., 2021). The emergence of bacteria resistant to various antibiotics and the high treatment costs associated with the resistance necessitates the development of novel, safe, efficient, and cost-effective natural medications (Hashempour-Baltork et al., 2019). Medicinal plants have become a prospect for discovering novel bioactive compounds, hence capable of

serving as possible antimicrobial agents and reducing the use of antibiotics in need to overcome the growth of MAR bacteria (Talib, 2011; Vaou et al., 2021).

Although various pharmacological have been studied from *S. alata*, there needs to be more experimental data available on the antibacterial activity of this plant against MAR bacteria, *Enterococcus* sp. and *Mammaliicoccus* sp. The study of phenolic and flavonoid contents in the leaves, stems, and roots of *S. alata* would also provide a better understanding of the plant's significance as a potential source for antioxidant agents. Besides, the compound separation would help to increase the purity of the isolated bioactive compounds upon characterisation. Thus, this study is intended to provide scientific evidence of *S. alata*'s antibacterial efficacy on MAR bacteria and its corresponding bioactive compounds, which can be exploited to manufacture health products from the plant.

1.3 Objectives

This research is conducted to achieve the following objectives:

i. To extract and characterise the extraction efficiency, total phenolic content and total flavonoid content in leaves, stems and roots of *S. alata* Linn. by using soxhlet extraction and ultrasonic extraction methods;

ii. To evaluate the antibacterial activities in leaves, stems and roots of *S. alata* Linn. against multiple antibiotic resistant bacteria, *Enterococcus* sp. and *Mammaliicoccus* sp.; and

iii. To separate and analyse the extracted bioactive compounds in the leaves, stems and roots of *S. alata* Linn. by using thin-layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) analysis.